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ORIGINAL ARTICLE

Multivariate morphometric study of *Apis florea* in Thailand

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SUMMARY

Morphometric analyses of *Apis florea* in Thailand were carried out in order to detect differences within this species. The nine body parts selected for analysis were: proboscis, antenna, forewing, hindwing, hind leg, the third and sixth sternites, and the third and fourth tergites. Twenty-two characters, consisting of widths, lengths or angles, were measured. Factor analysis sorted 14 characters of worker bees into four factors: (Factor 1) characters associated with size, hind leg and antenna; (Factor 2) length of wing venation and forewing; (Factor 3) number of hamuli and venation angle 37; and (Factor 4) venation angle 34. The results of factor and cluster analyses using the 22 characters revealed that the *A. florea* of Thailand are distributed as one group. Four characters (forewing radial cell length, metatarsus length, 3rd sternite length and antenna length) can be used to separate by Student-Newman-Keuls Statistics the *A. florea* of Samui and Pha-ngan Islands from the mainland.

Keywords: honey bees, *Apis florea*, morphometric analysis, Thailand

INTRODUCTION

Based on morphology, behaviour and geographic distribution, taxonomists have separated honey bees into nine species, five of which are found in Thailand. *Apis florea*, *A. andreniformis*, *A. dorsata*, and *A. cerana* are native species, whereas *A. mellifera* is introduced (Wongsiri, 1995). These bees are economically important in agriculture, medicine and the environment (Free, 1981). In addition, they are pollinators, especially *A. florea* which is an excellent pollinator of various economic crops and forest trees and has a wide foraging range (Wongsiri *et al.*, 2000). Most research on honey bee variation has been conducted on *A. mellifera* using morphometric analysis. Morphometrics is the measurement and statistical analysis of morphological structures of organisms (Daly, 1985). Few data on the native species of Thailand have been reported, especially on the morphometrics of *A. florea*. Rinderer *et al.* (1995) reported a comparison of *A. florea* and *A. andreniformis* for 44 morphometric characteristics for colonies from south eastern Thailand. Makhmoor & Ahmad (1998) reported studies of 16 morphological characters of 10 bees each of *A. florea*, *A. mellifera*, *A. cerana* and *A. dorsata* from the Jammu region of India. Narayanan *et al.* (1960) reported the tongue lengths and number of hamuli for 250 bees from five colonies at Pusa, India. Morphometric studies have been carried out on *A. cerana* in Thailand (Sylvester *et al.*, 1998) that showed that *A. cerana* can be separated into four geographic groups. In the present study, morphometric analyses of *A. florea* in Thailand were carried out in order to determine whether differences could be detected within this species, allowing separation into geographic groups.

MATERIALS AND METHODS

Fifteen worker bees were collected from each of fifty *Apis florea* colonies from different locations to sample the range of ecological variation in Thailand (table 1, fig. 1). These bees were preserved in 70% ethanol until they were dissected. The nine body parts selected were: proboscis, antenna, forewing, hindwing, hind leg, third and sixth sternites, and the third and fourth tergites. These parts were mounted on slides. A stereo microscope and the Dazzle Digital Video Creator and Digital Photo Marker program were used to take photos of all parts. Measurements of width, length or angle of 22 characters (Hepburn *et al.*, 2001; Ruttner, 1988) were carried out using the Image-Pro Plus program and recorded into a computer. Data were analysed by using factor and cluster analyses and one-way ANOVA.

RESULTS

The first statistical procedure performed a factor analysis on the colony means by using 22 characters for all 750 worker bees collected from the mainland and Samui and Pha-ngan Islands of Thailand. The means for all 750 worker bees are presented in table 2. Fourteen characters that had a high factor loading (greater than 0.6) were selected for further analysis: 3rd tergite length, 4th tergite length, femur length, 6th sternite length, tibia length, metatarsus length, 3rd sternite length, radial cell length, forewing length, apical portion of radial cell length, antenna length, venation angle 37, number of hamuli and venation angle 34. The second factor analysis using the colony means for the selected 14 characters grouped them into four factors with eigen values greater than one as new variables: (Factor 1) the characters associated with size, hind leg and antenna; (Factor 2) length of wing venation and forewing; (Factor 3) number of hamuli and venation angle 37; and (Factor 4) venation angle 34.

These factor score values were used in a cluster analysis in an attempt to classify the population structure of *A. florea* by mainland region, islands, and north and south of latitude 12°N in Thailand (at the Isthmus of Kra, the zoogeographic divide between central and south Thailand). The dendrogram shows that these bees were clumped into one group (fig. 2); that is, there was no discernible population structure based on region.

One-way ANOVA and multiple comparisons with Student-Newman-Keuls were used to test the mean value of the morphological characters in each region. All *F* tests indicated significant differences among the geographic/climatic groups except the test

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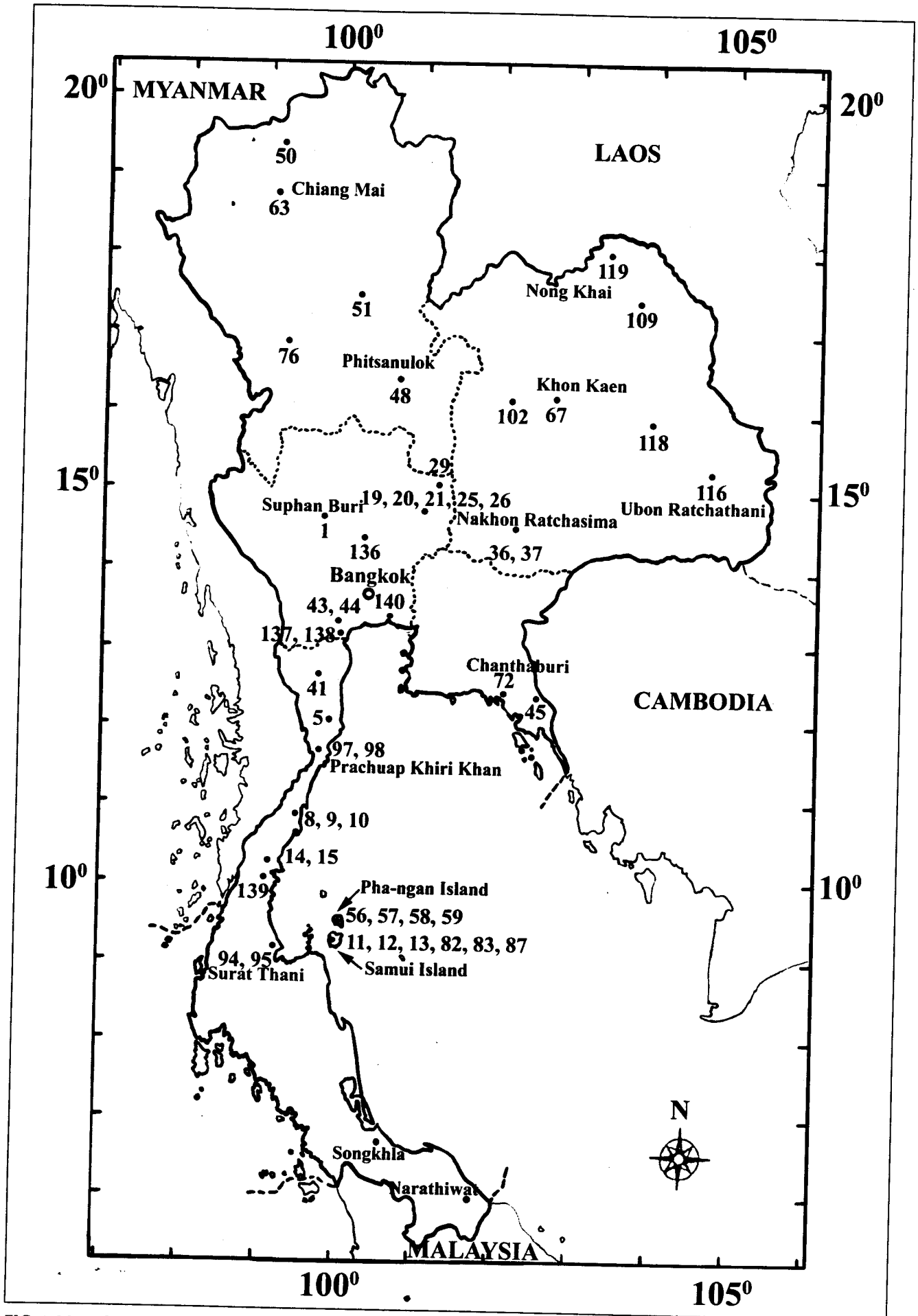

FIG. 1. Map of Thailand showing the sampling sites for *Apis florea*.

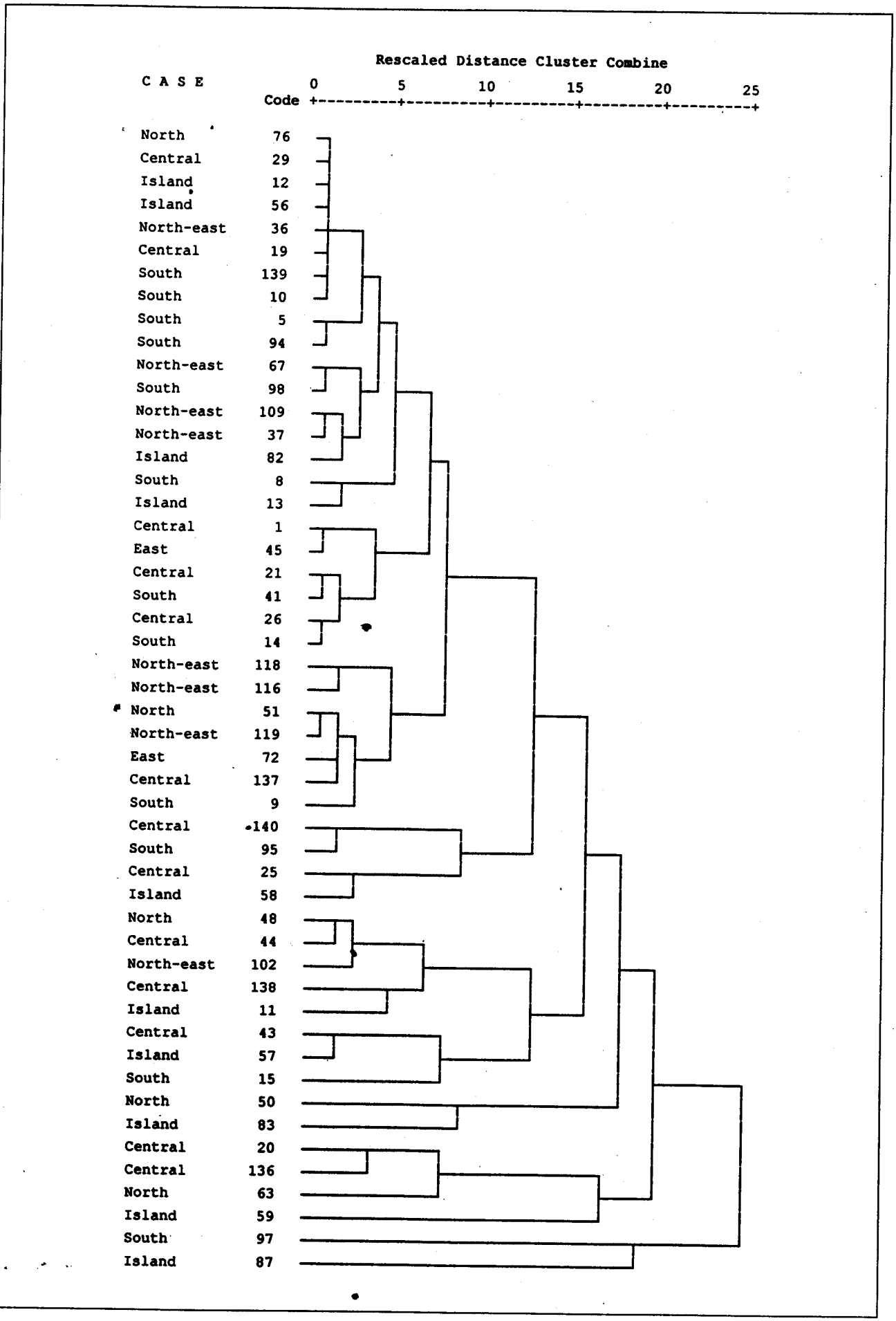

FIG. 2. Dendrogram showing the *Apis florea* samples classified by region.

TABLE 1. The locations of *Apis florea* sample collections.

Codes	Localities	Regions	Coordinates
1	Dan Chang	Changwat Suphan Buri	14° 49' 00N 99° 42' 00E
5	Sam Roi Yot	Changwat Prachuap Khiri Khan	12° 15' 00N 99° 52' 60E
8	Bang Saphan	Changwat Prachuap Khiri Khan	11° 11' 60N 99° 31' 00E
9	Bang Saphan	Changwat Prachuap Khiri Khan	11° 11' 60N 99° 31' 00E
10	Bang Saphan Noi	Changwat Prachuap Khiri Khan	11° 04' 60N 99° 28' 60E
11	Ko Samui	Samui Island, Changwat Surat Thani	09° 25' 60N 99° 58' 00E
12	Ko Samui	Samui Island, Changwat Surat Thani	09° 25' 60N 99° 58' 00E
13	Ko Samui	Samui Island, Changwat Surat Thani	09° 25' 60N 99° 58' 00E
14	Chumphon	Changwat Chumphon	10° 30' 00N 99° 10' 00E
15	Chumphon	Changwat Chumphon	10° 30' 00N 99° 10' 00E
19	Phatthana Nikhom	Changwat Lop Buri	14° 50' 12N 100° 59' 48E
20	Phatthana Nikhom	Changwat Lop Buri	14° 50' 12N 100° 59' 48E
21	Phatthana Nikhom	Changwat Lop Buri	14° 50' 12N 100° 59' 48E
25	Phatthana Nikhom	Changwat Lop Buri	14° 50' 12N 100° 59' 48E
26	Phatthana Nikhom	Changwat Lop Buri	14° 50' 12N 100° 59' 48E
29	Chai Badan	Changwat Lop Buri	15° 10' 23N 101° 05' 49E
36	Khon Buri	Changwat Nakhon Ratchasima	14° 31' 00N 102° 15' 00E
37	Khon Buri	Changwat Nakhon Ratchasima	14° 31' 00N 102° 15' 00E
41	Kaeng Krachan	Changwat Phetchaburi	12° 54' 00N 99° 34' 60E
43	Muang Samut Songkhram	Changwat Samut Songkhram	13° 22' 60N 99° 55' 60E
44	Muang Samut Songkhram	Changwat Samut Songkhram	13° 22' 60N 99° 55' 60E
45	Bo Rai	Changwat Trat	12° 26' 60N 102° 37' 00E
48	Noen Maprang	Changwat Phitsanulok	16° 34' 00N 100° 37' 60E
50	Chiang Dao	Changwat Chiang Mai	19° 22' 00N 98° 58' 00E
51	Muang Uttaradit	Changwat Uttaradit	17° 37' 52N 100° 06' 03E
56	Ko Phangan	Pha-ngan Island, Changwat Surat Thani	09° 43' 00N 100° 00' 00E
57	Ko Phangan	Pha-ngan Island, Changwat Surat Thani	09° 43' 00N 100° 00' 00E
58	Ko Phangan	Pha-ngan Island, Changwat Surat Thani	09° 43' 00N 100° 00' 00E
59	Ko Phangan	Pha-ngan Island, Changwat Surat Thani	09° 43' 00N 100° 00' 00E
63	Mae Rim	Changwat Chiang Mai	18° 54' 44N 98° 56' 23E
67	Phra Yuh	Changwat Khon Kaen	16° 19' 60N 102° 39' 00E
72	Laem Sing	Changwat Chanthaburi	12° 28' 60N 102° 04' 00E
76	Ban Tak	Changwat Tak	17° 02' 26N 99° 03' 47E
82	Ko Samui	Samui Island, Changwat Surat Thani	09° 33' 00N 99° 55' 60E
83	Ko Samui	Samui Island, Changwat Surat Thani	09° 33' 00N 99° 55' 60E
87	Ko Samui	Samui Island, Changwat Surat Thani	09° 25' 60N 99° 58' 00E
94	Chaiya	Changwat Surat Thani	09° 22' 60N 99° 13' 60E
95	Chaiya	Changwat Surat Thani	09° 22' 60N 99° 13' 60E
97	Muang Prachuap Khiri Khan	Changwat Prachuap Khiri Khan	11° 49' 00N 99° 47' 60E
98	Muang Prachuap Khiri Khan	Changwat Prachuap Khiri Khan	11° 49' 00N 99° 47' 60E
102	Phu Khieo	Changwat Chaiyaphum	16° 22' 00N 102° 07' 60E
109	Wanon Niwat	Changwat Sakon Nakhon	17° 37' 60N 103° 46' 00E
116	Muang Samsip	Changwat Ubon Ratchathani	15° 31' 00N 104° 43' 60E
118	Selaphum	Changwat Roi Et	16° 01' 60N 103° 57' 00E
119	So Phisai	Changwat Nong Khai	18° 04' 00N 103° 25' 60E
136	Wiset Chai Chan	Changwat Ang Thong	14° 34' 60N 100° 20' 60E
137	Damnoen Saduak	Changwat Ratchaburi	13° 31' 00N 99° 55' 60E
138	Damnoen Saduak	Changwat Ratchaburi	13° 31' 00N 99° 55' 60E
139	Pathiu	Changwat Chumphon	10° 41' 60N 99° 19' 00E
140	Phra Samut Chedi	Changwat Samut Prakan	13° 33' 00N 100° 34' 00E

on number of hamuli. It was found that the characters associated with abdominal size (3rd tergite length, 4th tergite length, 3rd sternite length and 6th sternite length) were smaller in the sample bees from Samui and Pha-ngan Islands. In addition, the characters associated with the appendages of bees from both islands (radial cell length, apical portion of radial cell length, metatarsus length and antenna length) were smaller than the mainland samples. Moreover, the characters radial cell length, metatarsus length, 3rd sternite length and antenna length were able to separate *A. florea* of these islands from the mainland. In addition, the comparative result of the mean value of antenna length separated samples from the east from the other regions. The comparative result of the mean value of metatarsus length separated samples from the central region from the other regions, but there were only a few samples from the east and north when compared to the other regions.

DISCUSSION

This morphometric study used 22 characters of *A. florea* distributed over different regions throughout the mainland and two of the islands of Thailand and found that the bees were not

TABLE 2. Means, standard deviations and coefficients of variation for 22 characters of 750 worker bees.

Character	Mean ± s.d.	CV
Tongue length	3.333 ± 0.150	4.506
Labial palp length	1.058 ± 0.033	3.134
Forewing length	6.293 ± 0.139	2.207
-radial cell length	2.318 ± 0.056	2.397
-apical position of radial cell length	1.459 ± 0.045	3.090
-venation angle 34	19.175 ± 1.308	6.823
-venation angle 35	99.602 ± 3.658	3.672
-venation angle 37	27.861 ± 2.390	8.580
Hindwing-basal portion of radial vein length	1.070 ± 0.043	3.995
-vannal lobe length	1.195 ± 0.063	5.231
-number of hamuli	11.173 ± 1.028	9.200
Femur length	1.721 ± 0.046	2.698
Tibia length	2.183 ± 0.060	2.749
Metatarsus length	1.254 ± 0.040	3.210
3rd tergite length	1.365 ± 0.055	3.997
Dark band of 4th tergite length	0.534 ± 0.079	14.706
4th tergite length	1.333 ± 0.048	3.580
3rd sternite length	1.777 ± 0.056	3.167
Wax mirror of 3rd sternite length	0.755 ± 0.044	5.870
6th sternite length	1.450 ± 0.037	2.532
Flagellum length	1.774 ± 0.118	6.633
Antenna length	2.819 ± 0.087	3.101

different using factor and cluster analyses. However, *A. florea* from Samui and Pha-ngan Islands tended to separate from the mainland samples by Student-Newman-Keuls statistics.

Fifty colonies of *A. florea* were classified by cluster analysis. It was found that 48 colonies were separated into the first group. However, two colonies, from Prachuap Khiri Khan (#97) and Samui Island (#87), were separated into a second group. One-way ANOVA and multiple comparisons with Student-Newman-Keuls were used to test the mean value of the morphological characters in each region. It was found that characters associated with the size of the sample bees from the islands (3rd tergite length, 4th tergite length, 3rd sternite length and 6th sternite length) were smaller than the mainland samples. This is consistent with Bergmann's rule that geographic races of one species are larger in the north than in the south (Ruttner, 1988). In addition, the characters associated with appendages of the sample bees from the islands (radial cell length, apical portion of radial cell length, metatarsus length and antenna length) were smaller than the mainland samples. Moreover only four characters (radial cell length, metatarsus length, 3rd sternite length and antenna length) separated *A. florea* of Samui and Pha-ngan Islands from the mainland samples by Student-Newman-Keuls Statistics. One explanation is that the island bees were the original bees or were introduced from the mainland by humans many years ago with the sea providing a reproductive barrier. However, significant differences were found only for a few characters, suggesting the separation between *A. florea* of the island and mainland samples in this study was not enough to classify island samples as a separate group.

Based on antenna length, samples of *A. florea* from the east in Thailand could be separated from the other regions, and based on metatarsus length samples from the central region could be separated also. But the quantity of *A. florea* samples from the east and north regions were few when compared to the other sampled regions. Thus, more samples should be studied to find a clearer conclusion.

We conclude that *A. florea* in Thailand exhibits less division into distinguishable geographic groups than does *A. cerana*, in which Sylvester *et al.* (1998) were able to distinguish four groups.

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